MycoKeys 84: 15–33 (2021)
doi: 10.3897/mycokeys.84.71701
https://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Characterization of *Diaporthe* species on *Camellia oleifera* in Hunan Province, with descriptions of two new species

Qin Yang[1,2], Jie Tang[1], Guo Y. Zhou[1,2]

1 *Forestry Biotechnology Hunan Key Laboratories, Central South University of Forestry and Technology, Changsha 410004, China* **2** *The Key Laboratory for Non-Wood Forest Cultivation and Conservation of the Ministry of Education, Central South University of Forestry and Technology, Changsha 410004, China*

Corresponding author: Guo Y. Zhou (zgyingqq@163.com)

Academic editor: R. Phookamsak | Received 16 July 2021 | Accepted 14 September 2021 | Published 18 October 2021

Citation: Yang Q, Tang J, Zhou GY (2021) Characterization of *Diaporthe* species on *Camellia oleifera* in Hunan Province, with descriptions of two new species. MycoKeys 84: 15–33. https://doi.org/10.3897/mycokeys.84.71701

Abstract

Tea-oil tree (*Camellia oleifera* Abel.) is an important edible oil woody plant with a planting area over 3,800,000 hectares in southern China. Species of *Diaporthe* inhabit a wide range of plant hosts as plant pathogens, endophytes and saprobes. At present, relatively little is known about the taxonomy and genetic diversity of *Diaporthe* on *C. oleifera*. Here, we conducted an extensive field survey in Hunan Province in China to identify and characterise *Diaporthe* species associated with tea-oil leaf spots. As a result, eleven isolates of *Diaporthe* were obtained from symptomatic *C. oleifera* leaves. These isolates were studied by applying a polyphasic approach including morphological and phylogenetic analyses of partial ITS, *cal*, *his3*, *tef1* and *tub2* gene regions. Two new *Diaporthe* species (*D. camelliae-oleiferae* and *D. hunanensis*) were proposed and described herein, and *C. oleifera* was revealed to be new host records of *D. hubeiensis* and *D. sojae*. This study indicated there is a potential of more undiscovered *Diaporthe* species from *C. oleifera* in China.

Keywords

Camellia oleifera, DNA phylogeny, systematics, taxonomy, two new taxa

Introduction

Tea-oil tree, *Camellia oleifera* Abel., is a unique woody edible oil species in China, mainly distributed in the Qinling-Huaihe River area. It has a long history of cultivation and utilization for more than 2300 years since ancient China (Zhuang 2008). Camellia oil, obtained from *C. oleifera* seeds, is rich in unsaturated fatty acids and unique flavors, and has become a rising high-quality edible vegetable oil in China. The edible of tea-oil is also conducive to preventing cardiovascular sclerosis, anti-tumor, lowering blood lipid, protecting liver and enhancing human immunity (Wang et al. 2007). Hunan Province leads the country in *C. oleifera* production with the average of 3.3~40,000 hm^2 to expand the cultivation area every year (Tan et al. 2018). By the end of 2017, the cultivation area of *C. oleifera* reached 1.4 million hm^2, tea oil 290100 tons, and output value of 35 billion yuan (Tan et al. 2018). Thus, the development of *C. oleifera* industry is of great significance for the economic development of Hunan Province and the poverty alleviation of local farmers.

Diseases are a major constraint to *C. oleifera* production. Anthracnose disease caused by *Colletotrichum* species is one of the foremost diseases in southern China, which can infect leaves and fruits of *C. oleifera*, causing up to 40% fruit drop and up to 40% camellia seeds loss (Wang et al. 2020). During July and August of 2020, new leaf spots were detected on tea-oil tree with irregular, brownish-grey lesions, often associated with leaf margins. Infected leaves cultured on medium had dark pycnidia producing ellipsoid guttulate conidia, similar to that of *Diaporthe* species (Yang et al. 2020, 2021). *Diaporthe* species are responsible for diseases on a wide range of plant hosts, including agricultural crops, forest trees and ornamentals, some of which can cause substantial yield losses (Santos et al. 2011; Gomes et al. 2013; Udayanga et al. 2015; Gao et al. 2016; Guarnaccia and Crous 2017, 2018; Yang et al. 2018, 2020, 2021). For instance, *D. ampelina*, the causal agent of Phomopsis cane and leaf spot, is known as a severe pathogen of grapevines (Hewitt and Pearson 1988), infecting all green tissues and causing yield reductions of up to 30% in temperate regions (Erincik et al. 2001). *Diaporthe citri* is another well-known pathogen exclusively found on *Citrus* spp. causing melanose, stem-end rot and gummosis in all the citrus production area except Europe (Mondal et al. 2007; Udayanga et al. 2014a; Guarnaccia and Crous 2017, 2018).

Species identification criteria in *Diaporthe* has mainly relied on host association, morphology and culture characteristics (Mostert et al. 2001; Santos and Phillips 2009; Udayanga et al. 2011), which resulted in the description of over 200 species. Some species of *Diaporthe* were reported to colonise a single host plant, while other species were found to be associated with different host plants (Santos and Phillips 2009; Diogo et al. 2010; Santos et al. 2011; Gomes et al. 2013). In addition, considerable variability of the phenotypic characters was found to be present within a species (Rehner and Uecker 1994; Mostert et al. 2001; Udayanga et al. 2011). During the past decade, a polyphasic approach, based on multi-locus DNA data, morphological, phytopathological and phylogenetical analyses, has been employed for species boundaries in the

genus *Diaporthe* (Huang et al. 2015; Gao et al. 2016, 2017; Guarnaccia and Crous 2017; Guarnaccia et al. 2018; Yang et al. 2018, 2020, 2021).

The classification of *Diaporthe* has been ongoing; however, little is known about species able to infect *C. oleifera*. Thus, the objective of the present study was to identify the prevalence of *Diaporthe* spp. associated with tea-oil tree leaf spot in the major plantations in Hunan Province based on morphological and phylogenetic features.

Materials and methods

Fungal isolation

Leaves of *C. oleifera* with typical symptoms of leaf spots were collected from the main tea-oil camellia production fields in Hunan Province. Small sections (3 × 3 mm) were cut from the margins of infected tissues, and surface-sterilised in 75% ethanol for 30 s, then sterilised in 5% sodium hypochlorite for 1 min, followed by three rinses with sterilised water and finally dried on sterilised filter paper. The sections were then plated on to PDA plates and incubated at 25 °C. Fungal growth was examined daily for up to 7 d. Isolates were then transferred aseptically to fresh PDA and purified by single-spore culturing. All fungal isolates were placed on PDA slants and stored at 4 °C. Specimens and axenic cultures are maintained in the Central South University of Forestry and Technology (CSUFT).

Morphological and cultural characterization

Agar plugs (6 mm diam.) were taken from the edge of actively growing cultures on PDA and transferred on to the centre of 9 cm diam. Petri dishes containing 2% tap water agar supplemented with sterile pine needles (PNA; Smith et al. 1996) and potato dextrose agar (PDA), and incubated at 25 °C under a 12 h near-ultraviolet light/12 h dark cycle to induce sporulation as described in recent studies (Gomes et al. 2013; Lombard et al. 2014). Colony characters and pigment production on PNA and PDA were noted after 10 d. Colony colours were rated according to Rayner (1970). Cultures were examined periodically for the development of ascomata and conidiomata. The morphological characteristics were examined by mounting fungal structures in clear lactic acid and 30 measurements at ×1000 magnification were determined for each isolate using a Leica compound microscope (DM 2500) with interference contrast (DIC) optics. Descriptions, nomenclature and illustrations of taxonomic novelties are deposited in MycoBank (Crous et al. 2004a).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from colonies grown on cellophane-covered PDA using a CTAB [cetyltrimethylammonium bromide] method (Doyle and Doyle 1990).

DNA was estimated by electrophoresis in 1% agarose gel, and the quality was measured using the NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA), following the user manual (Desjardins et al. 2009). PCR amplifications were performed in a DNA Engine Peltier Thermal Cycler (PTC-200; Bio-Rad Laboratories, Hercules, CA, USA). The primer set ITS1/ITS4 (White et al. 1990) was used to amplify the ITS region. The primer pair CAL228F/CAL737R (Carbone and Kohn 1999) was used to amplify the calmodulin gene (*cal*), and the primers CYLH4F (Crous et al. 2004b) and H3-1b (Glass and Donaldson 1995) were used to amplify part of the histone H3 (*his3*) gene. The primer pair EF1-728F/EF1-986R (Carbone and Kohn 1999) was used to amplify a partial fragment of the translation elongation factor 1-α gene (*tef1*). The primer set T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995) was used to amplify the beta-tubulin gene (*tub2*); the additional combination of Bt2a/Bt2b (Glass and Donaldson 1995) was used in case of amplification failure of the T1/Bt2b primer pair. The PCR amplifications of the genomic DNA with the phylogenetic markers were done using the same primer pairs and conditions as in Yang et al. (2018). PCR amplification products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL DNA Analyzer with a BigDye Terminater Kit v.3.1 (Invitrogen, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Phylogenetic analyses

The quality of the amplified nucleotide sequences was checked and combined using SeqMan v.7.1.0 and reference sequences were retrieved from the National Center for Biotechnology Information (NCBI), based on recent publications on the genus *Diaporthe* (Guarnaccia et al. 2018; Yang et al. 2018, 2020, 2021). Sequences were aligned using MAFFT v. 6 (Katoh and Toh 2010) and corrected manually using Bioedit 7.0.9.0 (Hall 1999). The best-fit nucleotide substitution models for each gene were selected using jModelTest v. 2.1.7 (Darriba et al. 2012) under the Akaike Information Criterion.

The phylogenetic analyses of the combined gene regions were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML was conducted using PhyML v. 3.0 (Guindon et al. 2010), with 1000 bootstrap replicates while BI was performed using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.0 (Ronquist et al. 2003). Two MCMC chains, started from random trees for 1,000,000 generations and trees, were sampled every 100th generation, resulting in a total of 10,000 trees. The first 25% of trees were discarded as burn-in of each analysis. Branches with significant Bayesian Posterior Probabilities (BPP) were estimated in the remaining 7500 trees. Phylogenetic trees were viewed with FigTree v.1.3.1 (Rambaut and Drummond 2010) and processed by Adobe Illustrator CS5. The nucleotide sequence data of the new taxa were deposited in GenBank (Table 1). The multilocus sequence alignments were deposited in TreeBASE (www.treebase.org) as accession S28703 and S22703.

Table 1. Isolates and GenBank accession numbers used in the phylogenetic analyses of *Diaporthe*.

Species	Isolate	Host	Location	GenBank accession numbers				
				ITS	*cal*	*his3*	*tef1*	*tub2*
D. acericola	MFLUCC 17-0956	*Acer negundo*	Italy	KY964224	KY964137	NA	KY964180	KY964074
D. acerigena	CFCC 52554	*Acer tataricum*	China	MH121489	MH121413	MH121449	MH121531	NA
D. alangii	CFCC 52556	*Alangium kurzii*	China	MH121491	MH121415	MH121451	MH121533	MH121573
D. alnea	CBS 146.46	*Alnus* sp.	Netherlands	KC343008	KC343250	KC343492	KC343734	KC343976
D. amygdali	CBS 126679	*Prunus dulcis*	Portugal	KC343022	KC343264	KC343506	AY343748	KC343990
D. angelicae	CBS 111592	*Heracleum sphondylium*	Austria	KC343027	KC343269	KC343511	KC343753	KC343995
D. apiculatum	CGMCC 3.17533	*Camellia sinensis*	China	KP267896	NA	NA	KP267970	KP293476
D. arecae	CBS 161.64	*Areca catechu*	India	KC343032	KC343274	KC343516	KC343758	KC344000
D. arengae	CBS 114979	*Arenga enngleri*	Hong Kong	KC343034	KC343276	KC343518	KC343760	KC344002
D. aseana	MFLUCC 12-0299	Unknown dead leaf	Thailand	KT459414	KT459464	NA	KT459448	KT459432
D. biguttulata	CGMCC 3.17248	*Citrus limon*	China	KJ490582	NA	KJ490524	KJ490461	KJ490403
	CFCC 52584	*Juglans regia*	China	MH121519	MH121437	MH121477	MH121561	MH121598
D. camelliae-oleiferae	**HNZZ027**	***Camellia oleifera***	**China**	**MZ509555**	**MZ504685**	**MZ504696**	**MZ504702**	**MZ504718**
	HNZZ030	***Camellia oleifera***	**China**	**MZ509556**	**MZ504686**	**MZ504697**	**MZ504708**	**MZ504719**
	HNZZ032	***Camellia oleifera***	**China**	**MZ509557**	**MZ504687**	**MZ504698**	**MZ504709**	**MZ504720**
D. celeris	CPC 28262	*Vitis vinifera*	Czech Republic	MG281017	MG281712	MG281363	MG281538	MG281190
D. celastrina	CBS 139.27	*Celastrus* sp.	USA	KC343047	KC343289	KC343531	KC343773	KC344015
D. cercidis	CFCC 52565	*Cercis chinensis*	China	MH121500	MH121424	MH121460	MH121542	MH121582
D. charlesworthii	BRIP 54884m	*Rapistrum rugostrum*	Australia	KJ197288	NA	NA	KJ197250	KJ197268
D. chrysalidocarpi	SAUCC194.35	*Chrysalidocarpus lutescens*	China	MT822563	MT855646	MT855532	MT855876	MT855760
D. cinnamomi	CFCC 52569	*Cinnamomum* sp.	China	MH121504	NA	MH121464	MH121546	MH121586
D. citriasiana	CGMCC 3.15224	*Citrus unshiu*	China	JQ954645	KC357491	KJ490515	JQ954663	KC357459
D. citrichinensis	CGMCC 3.15225	*Citrus* sp.	China	JQ954648	KC357494	NA	JQ954666	NA
D. collariana	MFLU 17-2770	*Magnolia champaca*	Thailand	MG806115	MG783042	NA	MG783040	MG783041
D. conica	CFCC 52571	*Alangium chinense*	China	MH121506	MH121428	MH121466	MH121548	MH121588
D. cucurbitae	CBS 136.25	*Arctium* sp.	Unknown	KC343031	KC343273	KC343515	KC343757	KC343999
D. cuppatea	CBS 117499	*Aspalathus linearis*	South Africa	KC343057	KC343299	KC343541	KC343783	KC344025
D. discoidispora	ZJUD89	*Citrus unshiu*	China	KJ490624	NA	KJ490566	KJ490503	KJ490445
D. drenthii	BRIP 66524	*Macadamia* sp.	South Africa	MN708229	NA	NA	MN696526	MN696537
D. endophytica	CBS 133811	*Schinus terebinthifolius*	Brazil	KC343065	KC343307	KC343549	KC343791	KC343065
D. eres	AR5193	*Ulmus* sp.	Germany	KJ210529	KJ434999	KJ420850	KJ210550	KJ420799
D. fraxini-angustifoliae	BRIP 54781	*Fraxinus angustifolia*	Australia	JX862528	NA	NA	JX862534	KF170920
D. fraxinicola	CFCC 52582	*Fraxinus chinensis*	China	MH121517	MH121435	NA	MH121559	NA
D. fructicola	MAFF 246408	*Passiflora edulis* × *P. edulis* f. *flavicarpa*	Japan	LC342734	LC342738	LC342737	LC342735	LC342736
D. fusicola	CGMCC 3.17087	*Lithocarpus glabra*	China	KF576281	KF576233	NA	KF576256	KF576305

Species	Isolate	Host	Location	GenBank accession numbers				
				ITS	*cal*	*his3*	*tef1*	*tub2*
D. ganzhouensis	CFCC 53087	Unknown	China	MK432665	MK442985	MK443010	MK578139	MK578065
D. garethjonesii	MFLUCC 12-0542a	Unknown dead leaf	Thailand	KT459423	KT459470	NA	KT459457	KT459441
D. guangxiensis	JZB320094	*Vitis vinifera*	China	MK335772	MK736727	NA	MK523566	MK500168
D. helicis	AR5211	*Hedera helix*	France	KJ210538	KJ435043	KJ420875	KJ210559	KJ420828
D. heterostemmatis	SAUCC194.85	*Heterostemma grandiflorum*	China	MT822613	MT855692	MT855581	MT855925	MT855810
D. hubeiensis	JZB320123	*Vitis vinifera*	China	MK335809	MK500235	NA	MK523570	MK500148
	HNZZ009	***Camellia oleifera***	**China**	**MZ509553**	**MZ504683**	**MZ504694**	**MZ504705**	**MZ504716**
	HNZZ019	***Camellia oleifera***	**China**	**MZ509554**	**MZ504684**	**MZ504695**	**MZ504706**	**MZ504717**
D. hunanensis	**HNZZ023**	***Camellia oleifera***	**China**	**MZ509550**	**MZ504680**	**MZ504691**	**MZ504702**	**MZ504713**
	HNZZ025	***Camellia oleifera***	**China**	**MZ509551**	**MZ504681**	**MZ504692**	**MZ504703**	**MZ504714**
	HNZZ033	***Camellia oleifera***	**China**	**MZ509552**	**MZ5046802**	**MZ504693**	**MZ504704**	**MZ504715**
D. kadsurae	CFCC 52586	*Kadsura longipedunculata*	China	MH121521	MH121439	MH121479	MH121563	MH121600
D. litchicola	BRIP 54900	*Litchi chinensis*	Australia	JX862533	NA	NA	JX862539	KF170925
D. lonicerae	MFLUCC 17-0963	*Lonicera* sp.	Italy	KY964190	KY964116	NA	KY964146	KY964073
D. masirevicii	BRIP 57892a	*Helianthus annuus*	Australia	KJ197277	NA	NA	KJ197239	KJ197257
D. miriciae	BRIP 54736j	*Helianthus annuus*	Australia	KJ197282	NA	NA	KJ197244	KJ197262
D. momicola	MFLUCC 16-0113	*Prunus persica*	China	KU557563	KU557611	NA	KU557631	KU55758
D. musigena	CBS 129519	*Musa* sp.	Australia	KC343143	KC343385	KC343627	KC343869	KC344111
D. neilliae	CBS 144.27	*Spiraea* sp.	USA	KC343144	KC343386	KC343628	KC343870	KC344112
D. nobilis	CBS 113470	*Castanea sativa*	Korea	KC343146	KC343388	KC343630	KC343872	KC344114
D. oraccinii	CGMCC 3.17531	*Camellia sinensis*	China	KP267863	NA	KP293517	KP267937	KP293443
D. ovoicicola	CGMCC 3.17093	*Citrus* sp.	China	KF576265	KF576223	NA	KF576240	KF576289
D. pandanicola	MFLU 18-0006	*Pandanus* sp.	Thailand	MG646974	NA	NA	NA	MG646930
D. pascoei	BRIP 54847	*Persea americana*	Australia	JX862532	NA	NA	JX862538	KF170924
D. passifloricola	CBS 141329	*Passiflora foetida*	Malaysia	KX228292	NA	KX228367	NA	KX228387
D. penetriteum	CGMCC 3.17532	*Camellia sinensis*	China	KP714505	NA	KP714493	KP714517	KP714529
D. perseae	CBS 151.73	*Persea gratissima*	Netherlands	KC343173	KC343415	KC343657	KC343899	KC344141
D. pescicola	MFLUCC 16-0105	*Prunus persica*	China	KU557555	KU557603	NA	KU557623	KU557579
D. pseudomangiferae	CBS 101339	*Mangifera indica*	Dominican Republic	KC343181	KC343423	KC343665	KC343907	KC344149
D. pseudophoenicicola	CBS 462.69	*Phoenix dactylifera*	Spain	KC343184	KC343426	KC343668	KC343910	KC344152
D. pulla	CBS 338.89	*Hedera helix*	Yugoslavia	KC343152	KC343394	KC343636	KC343878	KC344120
D. racemosae	CBS 143770	*Euclea racemosa*	South Africa	MG600223	MG600219	MG600221	MG600225	MG600227
D. schimae	CFCC 53103	*Schima superba*	China	MK432640	MK442962	MK442987	MK578116	MK578043
D. schini	CBS 133181	*Schinus terebinthifolius*	Brazil	KC343191	KC343433	KC343675	KC343917	KC344159
D. schoeni	MFLU 15-1279	*Schoenus nigricans*	Italy	KY964226	KY964139	NA	KY964182	KY964109
D. searlei	BRIP 66528	*Macadamia* sp.	South Africa	MN708231	NA	NA	NA	MN696540

Species	Isolate	Host	Location	GenBank accession numbers				
				ITS	*cal*	*his3*	*tef1*	*tub2*
D. sennicola	CFCC 51634	*Senna bicapsularis*	China	KY203722	KY228873	KY228879	KY228883	KY228889
D. siamensis	MFLUCC 10-573a	*Dasymaschalon* sp.	Thailand	JQ619879	NA	NA	JX275393	JX275429
D. sojae	FAU635	*Glycine max*	USA	KJ590719	KJ612116	KJ659208	KJ590762	KJ610875
	HNZZ008	***Camellia oleifera***	**China**	**MZ509547**	**MZ504677**	**MZ504688**	**MZ504699**	**MZ504710**
	HNZZ010	***Camellia oleifera***	**China**	**MZ509548**	**MZ504678**	**MZ504689**	**MZ504700**	**MZ504711**
	HNZZ022	***Camellia oleifera***	**China**	**MZ509549**	**MZ504679**	**MZ504690**	**MZ504701**	**MZ504712**
D. spinosa	PSCG	*Pyrus pyrifolia*	China	MK626849	MK691129	MK726156	MK654811	MK691234
D. sterilis	CBS 136969	*Vaccinium corymbosum*	Italy	KJ160579	KJ160548	MF418350	KJ160611	KJ160528
D. subclavata	ICMP20663	*Citrus unshiu*	China	KJ490587	NA	KJ490529	KJ490466	KJ490408
D. subellipicola	MFLU 17-1197	on dead wood	China	MG746632	NA	NA	MG746633	MG746634
D. subordinaria	CBS 464.90	*Plantago lanceolata*	New Zealand	KC343214	KC343456	KC343698	KC343940	KC344182
D. taoicola	MFLUCC 16-0117	*Prunus persica*	China	KU557567	NA	NA	KU557635	KU557591
D. tectonae	MFLUCC 12-0777	*Tectona grandis*	Thailand	KU712430	KU749345	NA	KU749359	KU743977
D. tectonendophytica	MFLUCC 13-0471	*Tectona grandis*	Thailand	KU712439	KU749354	NA	KU749367	KU749354
D. tectonigena	MFLUCC 12-0767	*Tectona grandis*	Thailand	KU712429	KU749358	NA	KU749371	KU743976
D. terebinthifolii	CBS 133180	*Schinus terebinthifolius*	Brazil	KC343216	KC343458	KC343700	KC343942	KC344184
D. tibetensis	CFCC 51999	*Juglandis regia*	China	MF279843	MF279888	MF279828	MF279858	MF279873
D. tulliensis	BRIP 62248a	*Theobroma cacao*	Australia	KR936130	NA	NA	KR936133	KR936132
D. ukurunduensis	CFCC 52592	*Acer ukurunduense*	China	MH121527	MH121445	MH121485	MH121569	NA
D. unshiuensis	CGMCC 3.17569	*Citrus unshiu*	China	KJ490587	NA	KJ490529	KJ490408	KJ490466
	CFCC 52594	*Carya illinoensis*	China	MH121529	MH121447	MH121487	MH121571	MH121606
D. viniferae	JZB320071	*Vitis vinifera*	China	MK341551	MK500107	NA	MK500119	MK500112
D. xishuangbanica	CGMCC 3.18282	*Camellia sinensis*	China	KX986783	NA	KX999255	KX999175	KX999216
D. yunnanensis	CGMCC 3.18289	*Coffea* sp.	China	KX986796	KX999290	KX999267	KX999188	KX999228
Diaporthella corylina	CBS 121124	*Corylus* sp.	China	KC343004	KC343246	KC343488	KC343730	KC343972

Note: NA, not applicable. Strains in this study are marked in bold.

Results

Phylogenetic analyses

The five-gene sequence dataset (ITS, *cal*, *his3*, *tef1* and *tub2*) was analysed to infer the interspecific relationships within *Diaporthe*. The dataset consisted of 96 sequences including the outgroup taxon, *Diaporthella corylina* (CBS 121124). A total of 2520 characters including gaps (510 for ITS, 518 for *cal*, 533 for *his3*, 460 for *tef1* and 499 for *tub2*) were included in the phylogenetic analysis. The best nucleotide substitution

model for ITS, *his3* and *tub2* was TrN+I+G, while HKY+I+G was selected for both *cal* and *tef1*. The topologies resulting from ML and BI analyses of the concatenated dataset were congruent (Fig. 1). According to the phylogenetic tree, two known species, *D. hubeiensis* and *D. sojae*, were part of *Diaporthe*. *Diaporthe camelliae-oleiferae* and *D. hunanensis* are new to science based on the distinct and well-supported molecular phylogenetic placement with their closest described relatives. Phylogenetically, *D. camelliae-oleiferae* clustered together with *D. pandanicola* and *D. viniferae*. *Diaporthe hunanensis* clustered together with *D. chrysalidocarpi* and other species, including *D. drenthii*, *D. searlei* and *D. spinosa*.

Taxonomy

Diaporthe camelliae-oleiferae **Q. Yang, sp. nov.**
MycoBank No: 840451
Figure 2

Diagnosis. Distinguished from the phylogenetically closely-related species, *D. pandanicola* and *D. viniferae* based on DNA sequence data.

Etymology. Named after the host species, *Camellia oleifera*.

Description. Asexual morph: *pycnidia* on PDA 500–660 μm in diam., superficial, scattered on PDA, dark brown to black, globose, solitary, or clustered in groups of 3–5 pycnidia. Pale yellow conidial drops exuding from ostioles. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* (7.5–)10–14(–15.5) × 1.5–2.3 μm (n = 30), aseptate, cylindrical, straight, densely aggregated, terminal, slightly tapered toward the apex. *Alpha conidia* 5–6.5(–7.5) × 1.9–2.3 μm (n = 30), aseptate, hyaline, ellipsoidal to fusiform, biguttulate. *Beta conidia* (26.5–)28.5–31(–33) × 0.8–1.2 μm (n = 30), hyaline, aseptate, filiform, sinuous at one end, eguttulate.

Culture characters. Culture incubated on PDA at 25 °C, originally flat with white fluffy aerial mycelium, becoming brown to black in the centre, with yellowish-cream conidial drops exuding from the ostioles after 20 days.

Specimens examined. China. Hunan Province: Zhuzhou City, on leaves of *Camellia oleifera*, 27°2'41"N, 113°19'17"E, 14 Aug. 2020, *Q. Yang* (holotype CSUFT027; ex-type living culture: HNZZ027; other living cultures: HNZZ030 and HNZZ032).

Notes. Three isolates representing *D. camelliae-oleiferae* cluster in a well-supported clade (ML/BI=100/1) and appear most closely related to *D. pandanicola* on *Pandanus* sp. and *D. viniferae* on *Vitis vinifera*. *Diaporthe camelliae-oleiferae* can be distinguished from *D. pandanicola* based on ITS and *tub2* loci (24/462 in ITS and 11/401 in *tub2*); from *D. viniferae* based on ITS, *cal*, *tef1* and *tub2* loci (13/453 in ITS, 42/448 in *cal*, 7/339 in *tef1* and 26/402 in *tub2*). Morphologically, *D. camelliae-oleiferae* differs from *D. viniferae* in having shorter alpha conidia (5–6.5 μm vs. 5–8.3 μm) (Manawasinghe et al. 2019); from *D. pandanicola* in having narrower alpha conidia (1.9–2.3 μm vs. 2.5–3.2 μm) (Huang et al. 2021).

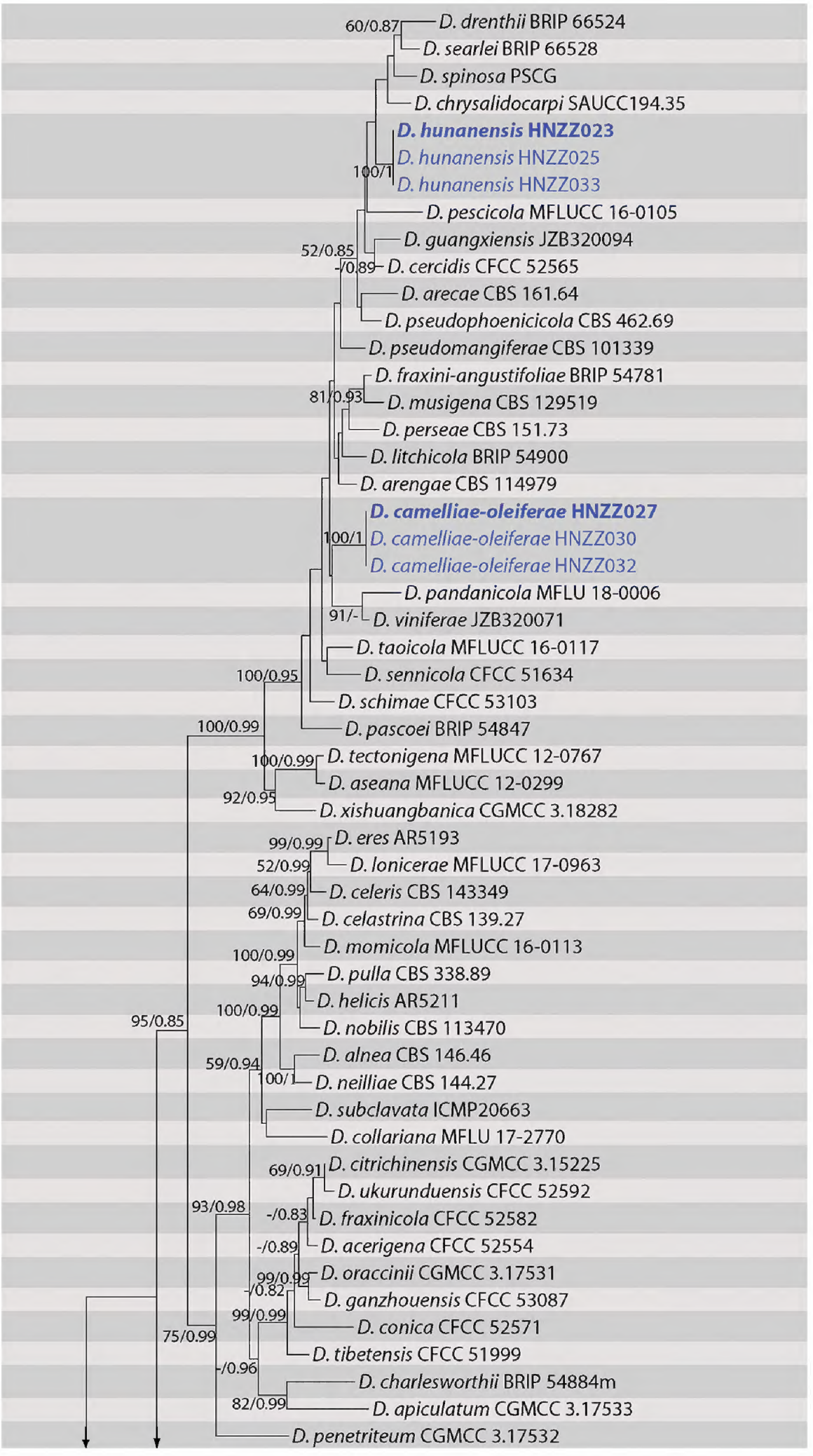

Figure 1. Phylogram of *Diaporthe* resulting from a maximum likelihood analysis based on combined ITS, *cal*, *his3*, *tef1* and *tub2*. Numbers above the branches indicate ML bootstraps (left, ML BS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.75). The tree is rooted with *Diaporthella corylina*. Isolates in current study are in blue. "-" indicates ML BS < 50% or BI PP < 0.75.

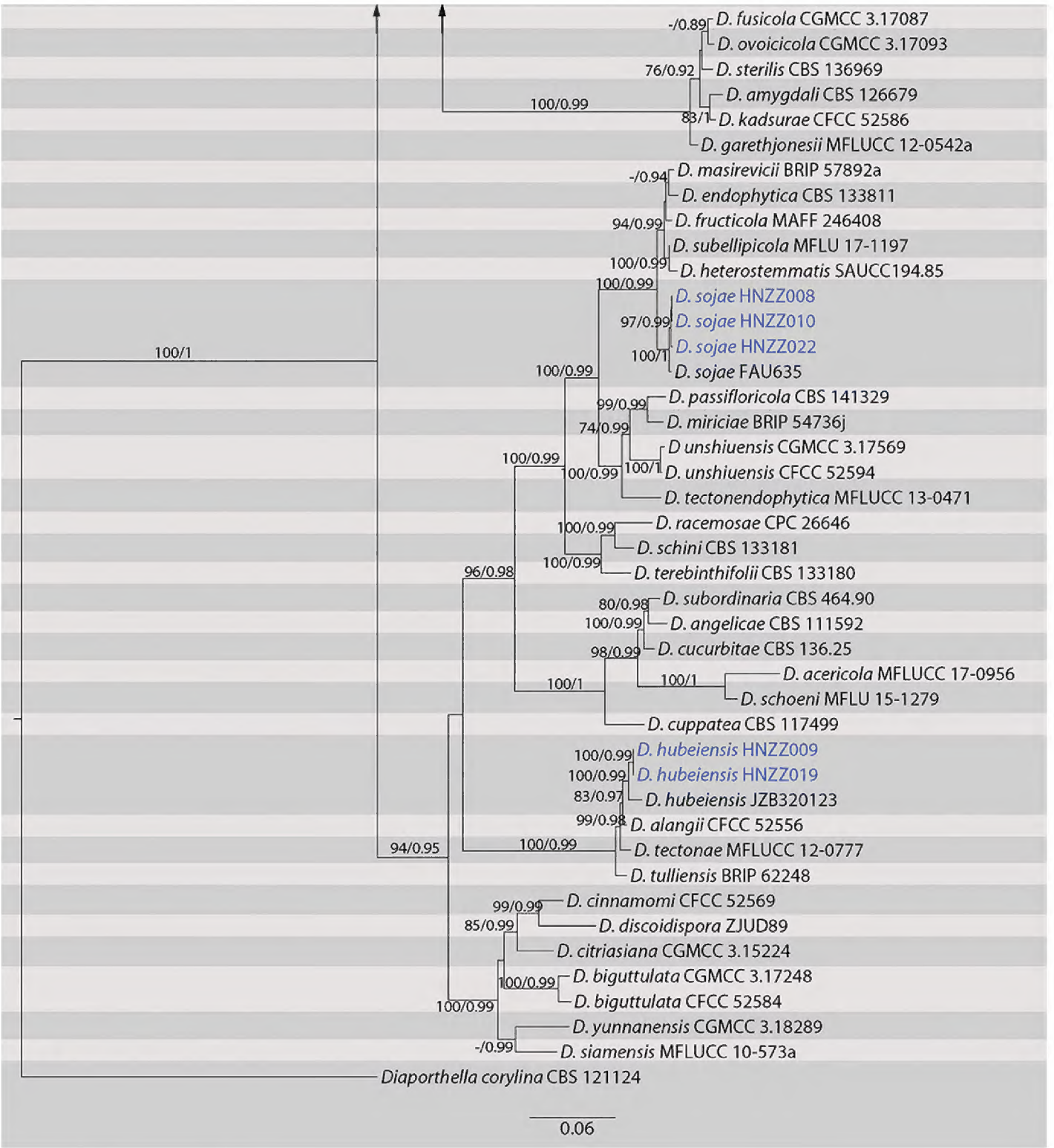

Figure 1. Continued

Diaporthe hubeiensis Dissanayake, X.H. Li & K.D. Hyde

Figure 3

Manawasinghe, Dissanayake, Li, Liu, Wanasinghe, Xu, Zhao, Zhang, Zhou, Hyde, Brooks & Yan, Frontiers in Microbiology 10(no. 1936): 20 (2019)

Description. Asexual morph: *pycnidia* on PDA in culture, 700–885 µm in diam., superficial, scattered, dark brown to black, globose or subglobose. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* (6.5–)7–10(–11.5) × 2–3.5 µm (n = 30), aseptate, cylindrical, phiailidic, straight or slightly curved. *Alpha conidia* 5.8–8(–8.5) × 2.5–3.2 µm (n = 30), aseptate, hyaline, ellipsoidal to cylindrical, biguttulate, blunt at both ends. *Beta conidia* not observed.

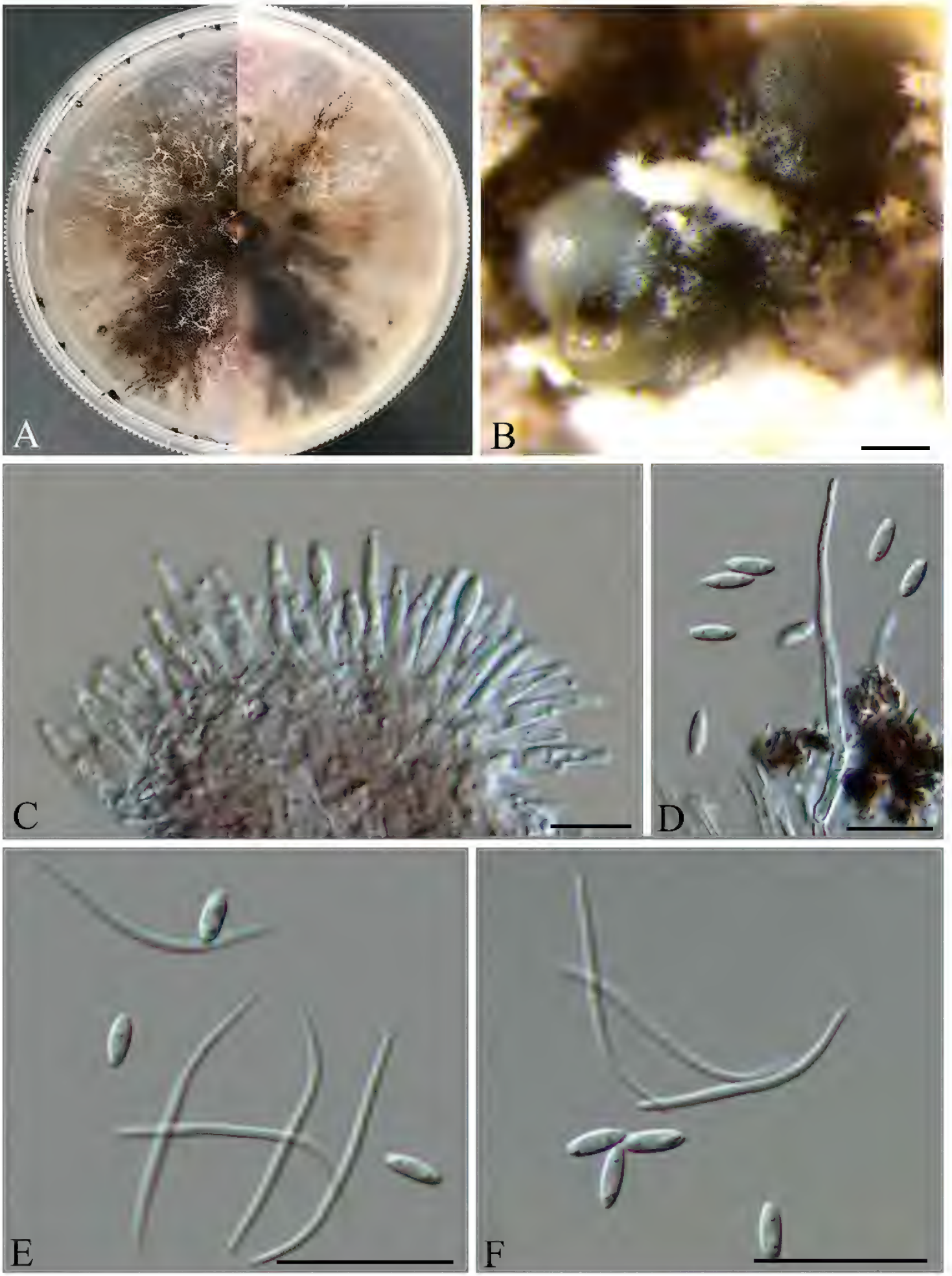

Figure 2. *Diaporthe camelliae-oleiferae* (HNZZ027) **A** Culture on PDA **B** conidiomata **C** conidiogenous cells **D–F** alpha and beta conidia. Scale bars: 200 μm (**B**); 10 μm (**C–D**); 20 μm (**E, F**).

Culture characters. Culture incubated on PDA at 25 °C, originally flat with white felted aerial mycelium, becoming dark brown mycelium due to pigment formation, conidiomata irregularly distributed over agar surface after 20 days.

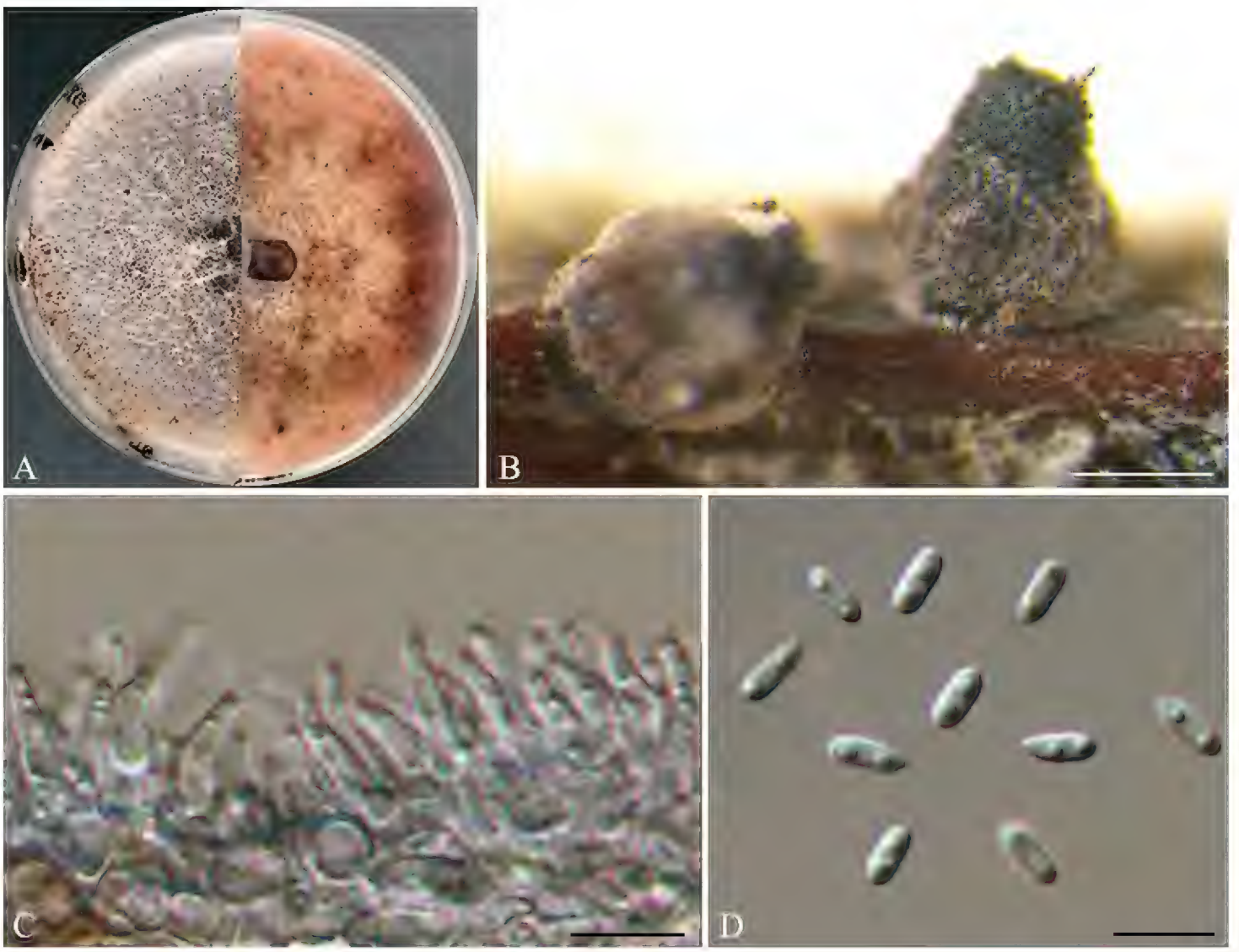

Figure 3. *Diaporthe hubeiensis* (HNZZ019) **A** Culture on PDA **B** conidiomata **C** conidiogenous cells **D** alpha conidia. Scale bars: 500 μm (**B**); 10 μm (**C–D**).

Specimens examined. China. Hunan Province: Zhuzhou City, on leaves of *Camellia oleifera*, 27°2'35"N, 113°19'20"E, 14 Aug. 2020, *Q. Yang* (CSUFT019; living cultures: HNZZ019 and HNZZ009).

Notes. *Diaporthe hubeiensis* was originally described as pathogen of grapevines in Hubei Province, China (Manawasinghe et al. 2019). In the present study, two isolates (HNZZ019 and HNZZ009) are closely related to *D. hubeiensis* in the combined phylogenetic tree (Fig. 1). The differences of nucleotides in the concatenated alignment (1/460 in ITS, 3/458 in *cal*, 1/320 in *his3* and 3/433 in *tub2*) are minor. Morphological comparison indicated that the isolates were similar to *D. hubeiensis* by the size of alpha conidia. We therefore identify the isolates as belonging to *D. hubeiensis*.

***Diaporthe hunanensis* Q. Yang, sp. nov.**
MycoBank No: 840452
Figure 4

Diagnosis. Distinguished from its phylogenetically closely-related species, *D. chrysalidocarpi*, *D. drenthii*, *D. searlei* and *D. spinosa* based on DNA sequence data.

Figure 4. *Diaporthe hunanensis* (HNZZ023) **A** Culture on PDA **B** conidiomata **C** conidiogenous cells **D** alpha conidia. Scale bars: 500 μm (**B**); 10 μm (**C–D**).

Etymology. In reference to the Hunan province, from where the fungus was first collected.

Description. Asexual morph: *pycnidia* on PDA 180–300 μm in diam., superficial, scattered, black, globose, solitary in most. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* (8–)9–15(–16.5) × 1.7–2.1 μm (n = 30), aseptate, cylindrical, phiailidic, straight or slightly curved. *Alpha conidia* 6.5–7.5(–8.5) × 2.4–2.9 μm (n = 30), aseptate, hyaline, ellipsoidal, biguttulate, both ends obtuse. *Beta conidia* not observed.

Culture characters. Culture incubated on PDA at 25 °C, originally flat with white fluffy aerial mycelium, becoming pale brown with age, with visible solitary conidiomata at maturity after 18 days.

Specimens examined. China. Hunan Province: Zhuzhou City, on leaves of *Camellia oleifera*, 27°2'41"N, 113°19'17"E, 14 Aug. 2020, *Q. Yang* (holotype CSUFT 023; ex-type living culture: HNZZ023; living cultures: HNZZ025 and HNZZ033).

Notes. Three isolates representing *D. hunanensis* cluster in a well-supported clade (ML/BI=100/1) and appear most closely related to *D. chrysalidocarpi* on *Chrysalidocarpus lutescens*, *D. drenthii* and *D. searlei* on *Macadamia* sp., and *D. spinosa* on *P. pyrifolia* cv. Cuiguan. *Diaporthe hunanensis* can be distinguished from *D. chrysalidocarpi* based on ITS, *cal*, *his3* and *tub2* loci (7/457 in ITS, 28/448 in *cal*, 8/455 in *his3* and 5/401 in *tub2*); from *D. drenthii* based on ITS, *tef1* and *tub2* loci (9/457 in ITS, 13/328 in *tef1* and 23/401 in *tub2*); from *D. searlei* based on ITS and *tub2* loci (10/457 in ITS and 12/401 in *tub2*); from *D. spinosa* based on ITS, *cal*, *his3*, *tef1* and *tub2* loci (8/458 in ITS, 31/448 in *cal*, 5/455 in *his3*, 8/328 in *tef1* and 19/401 in *tub2*). Morphologically, *D. chrysalidocarpi* produces only beta conidia, while *D. hunanensis* produces alpha conidia (Huang et al. 2021); *D. hunanensis* differs from *D. drenthii* and *D. searlei* in wider alpha conidia (2.4–2.9 μm in *D. hunanensis* vs. 1.5–2.5 μm in *D. drenthii* vs. 1.5–2 μm in *D. searlei*) (Wrona et al. 2020); from *D. spinosa* in shorter alpha conidia (6.5–7.5 × 2.4–2.9 μm vs. 5.5–8 × 2–3.5 μm) (Guo et al. 2020). Therefore, we establish this fungus as a novel species.

Diaporthe sojae **Lehman, Ann. Mo. bot. Gdn 10: 128 (1923)**
Figure 5

Description. Sexual morph: *perithecia* on pine needles in culture, black, globose, 250–500 μm in diam., densely clustered in groups, deeply immersed with elongated, tapering perithecial necks protruding through substrata, 525–800 μm. *Asci* unitunicate, 8-spored, sessile, elongate to clavate, (35–)37–42(–44.5) × (8–)10–11.5 μm (n = 30). *Ascospores* hyaline, two-celled, often 4-guttulate, with larger guttules at centre and smaller one at ends, elongated to elliptical, slightly or not constricted at septum, (9–)9.5–11.5 × 2.7–4 μm (n = 30). Asexual morph not observed.

Culture characters. Culture incubated on PNA at 25 °C, originally white, fluffy aerial mycelium, reverse yellowish pigmentation developing in centre, later becoming dark brown, with yellowish-cream drops exuding from the perithecia after 15 days.

Specimens examined. China. Hunan Province: Zhuzhou City, on leaves of *Camellia oleifera*, 27°2'41"N, 113°19'17"E, 14 Aug. 2020, *Q. Yang* (USUFT 022; living cultures: HNZZ022, HNZZ008 and HNZZ010).

Notes. *Diaporthe sojae* was first reported on pods and stems of soybean, and subsequently reported on a wide range of hosts (Dissanayake et al. 2015; Udayanga et al. 2015; Guo et al. 2020). It was also reported on some fruit trees in China, such as *Vitis* spp. (Dissanayake et al. 2015) and *Citrus* spp. (Huang et al. 2015). In the present, three isolates (HNZZ008, HNZZ010 and HNZZ022) are closely related to *D. sojae* in the combined phylogenetic tree (Fig. 1). The differences of nucleotides in the concatenated alignment (1/460 in ITS, 3/458 in *cal*, 1/320 in *his3* and 3/433 in *tub2*) are minor. Compared with the description of the ex-type isolate FAU635, the isolate has wider asci (10–11.5 μm vs. 7–9 μm) (Udayanga et al. 2015). We therefore identify the isolates as belonging to *D. sojae*.

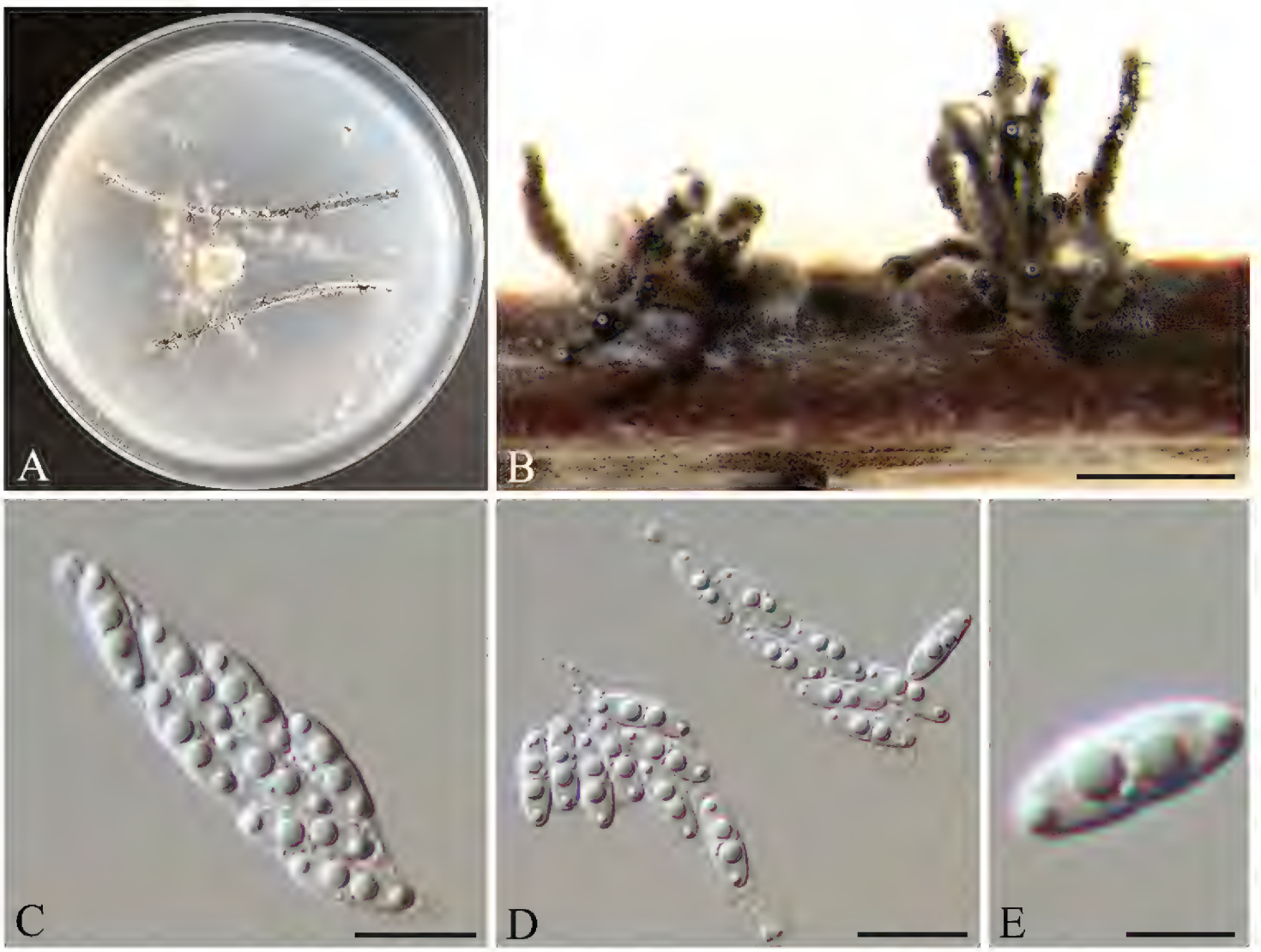

Figure 5. *Diaporthe sojae* (HNZZ022) **A** Culture on PNA **B** ascomata **C–E** asci and ascospores. Scale bars: 500 μm (**B**); 10 μm (**C–E**).

Discussion

In this study, an important oil-tea tree species, *Camellia oleifera* was investigated and *Camellia* leaf disease was found as a common disease in plantations in Hunan Province. Identification of our collections was conducted, based on isolates from symptomatic leaves of *C. oleifera* using five combined loci (ITS, *cal*, *his3*, *tef1* and *tub2*), as well as morphological characters. It includes *D. hubeiensis*, *D. sojae*, as well as two new species named *D. camelliae-oleiferae* and *D. hunanensis*.

The expanding cultivation of *C. oleifera* over the last several decades has attracted increasing attention from plant pathologists to infectious diseases on this crop. Therein, diseases caused by *Diaporthe* species have becoming the emerging Camellia leaf diseases in southern China (Gao et al. 2016; Guarnaccia et al. 2018; Yang et al. 2018; Zhou and Hou 2019). Understanding the diversity of *Diaporthe* species and the genetic variation within pathogen populations could help in developing sustainable disease management strategies.

According to the USDA Fungal–host interaction database, there are two records of *Diaporthe* species associated with *C. oleifera* (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm) (accessed 9 September 2021). These records are related

to the following two *Diaporthe* species: *D. eres* and *D. huangshanensis* (Zhou and Hou 2019). *Diaporthe eres*, the type species of the genus, was described by Nitschke (1870) on *Ulmus* sp. collected in Germany, which has a widespread distribution and a broad host range as pathogens, endophytes or saprobes (Udayanga et al. 2014b). *Diaporthe eres* differs from *D. camelliae-oleiferae* and *D. hunanensis* in having wider alpha conidia (3–4 μm in *D. eres* vs. 1.9–2.3 μm in *D. camelliae-oleiferae* vs. 2.4–2.9 μm in *D. hunanensis*) (Gomes et al. 2003); *D. huangshanensis* differs from *D. camelliae-oleiferae* in having larger alpha conidia (5.7–8.4 × 2.7–4.5 μm vs. 5–6.5 × 1.9–2.3 μm); from *D. hunanensis* in having wider alpha conidia (2.7–4.5 μm vs. 2.4–2.9 μm) and longer conidiophores (12.1–23.5 μm vs. 9–15 μm) (Zhou and Hou 2019).

As the species concept of *Diaporthe* has been improved a lot by using molecular data (Huang et al. 2015; Gao et al. 2016, 2017; Guarnaccia and Crous 2017; Guarnaccia et al. 2018; Yang et al. 2018, 2020, 2021; Manawasinghe et al. 2019; Guo et al. 2020), many new species have been discovered and reported in recent years. In this study, the *Diaporthe* isolates from *C. oleifera* were identified based on sequence analysis and morphological characteristics. Future studies should focus on pathogenicity, epidemiology and fungicide sensitivity of the important plant fungal pathogen to develop effective management of *C. oleifera* disease and on the pathogenic molecular mechanism.

Acknowledgements

This study is financed by the Research Foundation of Education Bureau of Hunan Province, China (Project No.: 19B608) and the introduction of talent research start-up fund project of CSUFT (Project No.: 2019YJ025).

References

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 3: 553–556. https://doi.org/10.2307/3761358

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004a) MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.

Crous PW, Groenewald JZ, Risède JM, Simoneau P, Hywel-Jones NL (2004b) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Studies in Mycology 50: 415–430.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772–772. https://doi.org/10.1038/nmeth.2109

Desjardins P, Hansen JB, Allen M (2009) Microvolume protein concentration determination using the NanoDrop 2000c spectrophotometer. Journal of Visualized Experiments: JoVE 33: 1–3. https://doi.org/10.3791/1610

Diogo E, Santos JM, Phillips AJ (2010) Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal. Fungal Diversity 44: 107–115. https://doi.org/10.1007/s13225-010-0057-x

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13–15. https://doi.org/10.2307/2419362

Erincik O, Madden L, Ferree D, Ellis M (2001) Effect of growth stage on susceptibility of grape berry and rachis tissues to infection by *Phomopsis viticola*. Plant Disease 85: 517–520. https://doi.org/10.1094/PDIS.2001.85.5.517

Gao YH, Liu F, Cai L (2016) Unravelling *Diaporthe* species associated with *Camellia*. Systematics and Biodiversity 14: 102–117. https://doi.org/10.1080/14772000.2015.1101027

Gao YH, Liu F, Duan W, Crous PW, Cai L (2017) *Diaporthe* is paraphyletic. IMA Fungus 8: 153–187. https://doi.org/10.5598/imafungus.2017.08.01.11

Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia 31: 1–41. https://doi.org/10.3767/003158513X666844

Guarnaccia V, Crous PW (2017) Emerging citrus diseases in Europe caused by species of *Diaporthe*. IMA Fungus 8: 317–334. https://doi.org/10.5598/imafungus.2017.08.02.07

Guarnaccia V, Crous PW (2018) Species of *Diaporthe* on *Camellia* and *Citrus* in the Azores Islands. Phytopathologia Mediterranea 57: 307–319.

Guarnaccia V, Groenewald JZ, Woodhall J, Armengol J, Cinelli T, Eichmeier A, Ezra D, Fontaine F, Gramaje D, Gutierrez-Aguirregabiria A, Kaliterna J, Kiss L, Larignon P, Luque J, Mugnai L, Naor V, Raposo R, Sándor E, Váczy KZ, Crous PW (2018) *Diaporthe* diversity and pathogenicity revealed from a broad survey of grapevine diseases in Europe. Persoonia 40: 135–153. https://doi.org/10.3767/persoonia.2018.40.06

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321. https://doi.org/10.1093/sysbio/syq010

Guo YS, Crous PW, Bai Q, Fu M, Yang MM, Wang XH, Du YM, Hong N, Xu WX, Wang GP (2020) High diversity of *Diaporthe* species associated with pear shoot canker in China. Persoonia 45: 132–162. https://doi.org/10.3767/persoonia.2020.45.05

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hewitt W, Pearson R (1988) Phomopsis cane and leaf spot. Compendium of grape diseases: 17–18. APS Press, St Paul, Minnesota.

Huang F, Udayanga D, Wang X, Hou X, Mei X, Fu Y, Hyde KD, Li HY (2015) Endophytic *Diaporthe* associated with *Citrus*: A phylogenetic reassessment with seven new species from China. Fungal Biology 119: 331–347. https://doi.org/10.1016/j.funbio.2015.02.006

Huang ST, Xia JW, Zhang XG, Sun WX (2021) Morphological and phylogenetic analyses reveal three new species of *Diaporthe* from Yunnan, China. MycoKeys 78: 49–77. https://doi.org/10.3897/mycokeys.78.60878

Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 26: 1899–1900. https://doi.org/10.1093/bioinformatics/btq224

Li H, Zhu XD, Liu JA, Xu JP (2014) Population genetic structure of *Colletotrichum gloeosporioides* ausing anthracnose of *Camellia oleifera* in China. Acta Phytopathologica Sinica 44: 620–628.

Lombard L, Van Leeuwen GCM, Guarnaccia V, Polizzi G, Van Rijswick PC, Karin Rosendahl KC, Gabler J, Crous PW (2014) *Diaporthe* species associated with *Vaccinium*, with specific reference to Europe. Phytopathologia Mediterranea 53: 287–299.

Manawasinghe IS, Dissanayake AJ, Li X, Liu M, Wanasinghe DN, Xu J, Zhao W, Zhang W, Zhou Y, Hyde KD, Brooks S, Yan J (2019) High genetic diversity and species complexity of *Diaporthe* associated with grapevine dieback in China. Frontiers in Microbiology 10: e1936. https://doi.org/10.3389/fmicb.2019.01936

Mondal SN, Vincent A, Reis RF, Timmer LW (2007) Saprophytic colonization of citrus twigs by *Diaporthe citri* and factors affecting pycnidial production and conidial survival. Plant Disease 91: 387–392. https://doi.org/10.1094/PDIS-91-4-0387

Mostert L, Crous PW, Kang JC, Phillips AJ (2001) Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. Mycologia 93: 146–167. https://doi.org/10.2307/3761612

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7: 103–116. https://doi.org/10.1006/mpev.1996.0376

Rambaut A, Drummond A (2010) FigTree v.1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute, Kew.

Rehner SA, Uecker FA (1994) Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Canadian Journal of Botany 72: 1666–1674. https://doi.org/10.1139/b94-204

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Santos JM, Phillips AJL (2009) Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. Fungal Diversity 34: 111–125.

Santos JM, Vrandečić K, Ćosić J, Duvnjak T, Phillips AJL (2011) Resolving the *Diaporthe* species occurring on soybean in Croatia. Persoonia 27: 9–19. https://doi.org/10.3767/003158511X603719

Smith H, Wingfeld MJ, Coutinho TA, Crous PW (1996) *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. South African Journal of Botany 62: 86–88. https://doi.org/10.1016/S0254-6299(15)30596-2

Tan XF, Guan TQ, Yuan J (2018) Report on upgrading output value of oil-tea industry to 100 billion RMB in Hunan. Non-wood Forest Research 36: 1–4.

Tibpromma S, Hyde KD, Bhat JD, Mortimer PE, Xu J, Promputtha I, Doilom M, Yang JB, Tang AMC, Karunarathna SC (2018) Identification of endophytic fungi from leaves of Pandanaceae based on their morphotypes and DNA sequence data from southern Thailand. MycoKeys 33: 25–67. https://doi.org/10.3897/mycokeys.33.23670

Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2014b) Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. Fungal Diversity 67: 203–229. https://doi.org/10.1007/s13225-014-0297-2

Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2015) The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. Fungal Biology 119: 383–407. https://doi.org/10.1016/j.funbio.2014.10.009

Udayanga D, Castlebury LA, Rossman AY, Hyde KD (2014a) Species limits in *Diaporthe*: molecular re-assessment of *D. citri*, *D. cytosporella*, *D. foeniculina* and *D. rudis*. Persoonia 32: 83–101. https://doi.org/10.3767/003158514X679984

Udayanga D, Liu X, McKenzie EH, Chukeatirote E, Bahkali AH, Hyde KD (2011) The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Diversity 50: 189–225. https://doi.org/10.1007/s13225-011-0126-9

Wang WJ, Chen CG, Cheng J (2007) The medicinal active role of tea oil in health care. Food and Nutrition in China 9: 48–51.

Wang Y, Chen JY, Xu XW, Cheng JY, Zheng L, Huang JB, Li DW (2020) Identification and characterization of *Colletotrichum* species associated with anthracnose disease of *Camellia oleifera* in China. Plant Disease 104: 474–482. https://doi.org/10.1094/PDIS-11-18-1955-RE

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18: 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wrona CJ, Mohankumar V, Schoeman MH, Tan YP, Shivas RG, Jeff-Ego OS, Akinsanmi OA (2020) Phomopsis husk rot of macadamia in Australia and South Africa caused by novel *Diaporthe* species. Plant Pathology 69: 911–921. https://doi.org/10.1111/ppa.13170

Yang Q, Fan XL, Guarnaccia V, Tian CM (2018) High diversity of *Diaporthe* species associated with dieback diseases in China, with twelve new species described. MycoKeys 39: 97–149. https://doi.org/10.3897/mycokeys.39.26914

Yang Q, Jiang N, Tian CM (2020) Three new *Diaporthe* species from Shaanxi Province, China. MycoKeys 67: 1–18. https://doi.org/10.3897/mycokeys.67.49483

Yang Q, Jiang N, Tian CM (2021) New species and records of *Diaporthe* from Jiangxi Province, China. MycoKeys 77: 41–64. https://doi.org/10.3897/mycokeys.77.59999

Zhou H, Hou CL (2019) Three new species of *Diaporthe* from China based on morphological characters and DNA sequence data analyses. Phytotaxa 422: 157–174. https://doi.org/10.11646/phytotaxa.422.2.3

Zhuang RL (2008) *Camellia oleifera*. 2nd Edn. China Forestry Press, Beijing.